The numerical values for the absolute and relative sizes of all these components are shown in Table I.

The most striking of this series of changes is the increase of the range of variability of the several components forming the body. At 6 lunar months there is a maximum difference of only 9% in their relative distribution. By birth there is a maximum difference of 40%. And at maturity there is a maximum difference of over 1000%. These computations are in terms of the smallest component in each series, but they may also be demonstrated in terms of the largest component or in terms of absolute values.

11261

Keto-Reacting Substances in the Bile of Dogs.

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A study of the literature revealed that certain investigators have identified and isolated various ketonic acids from the bile of different animals. Fernholz[1] isolated 3-hydroxy-6-ketocholanic acid from hog bile and studied its properties. Schoenheimer and others[2, 3] confirmed the isolation of this ketonic acid, and further stated that the ketonic acids of hog bile comprise approximately 10% of the total crude acids. Wieland and Kishi[4] isolated and identified 3-hydroxy-12-ketocholanic acid from ox bile. Sobotka found 3-hydroxy-12-ketocholanic acid in human bile.[5]

No one apparently has reported a method for the quantitative determination of carbonyl or keto-reacting substances in bile. Since our work required such a method, we have been fortunate at having at our disposal a quantitative method which had been developed by Dr. Gustus of the Wilson Laboratories, Chicago. The principle of this method is as follows: The carbonyl or keto groups in the bile are allowed to react with an excess of hydroxylamine. The remaining hydroxylamine is combined with diacetyl-monoxime converting the

1 Fernholz, E., *H.-S. Z. f. Physiol. Chem.*, 1935, **232**, 202.

2 Schoenheimer, R., and Johnston, C. G., *J. Biol. Chem.*, 1937, **120**, 499.

3 Anchel, M., and Schoenheimer, R., *J. Biol. Chem.*, 1938, **124**, 609.

4 Wieland, H., and Kishi, S., *H.-S. Z. f. Physiol. Chem.*, 1933, **214**, 47.

5 Sobotka, H., *Chem. Rev.*, 1934, **15**, 334.

latter to the dioxime compound. The dioxime is precipitated with nickel acetate, and the resulting precipitate washed, dried and weighed. By calculation, the amount of carbonyl groups that was originally present in the bile and that combined with the hydroxylamine can be obtained. The exact details of this analysis for carbonyl groups in bile will be published later by Dr. Gustus.

The accuracy of the method has been examined in two ways. First, the chemist was supplied with duplicate unknowns of the same bile obtained from 11 bile fistula dogs (Rous-McMaster method), some of which received no bile salts, and others, bile salts containing varying amounts of ketocholanic acid. The analyses of the duplicate unknowns checked within ±6% of the mean, except in one case in which the error was ±15%. Second, pure triketocholanic acid added to dogs' bile was recovered in amounts varying from 95% to 99%.

Control output of keto-reacting substances. Before ascertaining the effect of feeding natural and oxidized bile acids on the keto-reacting substances in bile, it was necessary to determine the amount of keto-reacting substances present in "control" bile. By "control" bile is meant the bile that is secreted when the animal is fed the standard diet only and no bile or bile salts are administered. This was done in 55 tests on 15 dogs. The average control daily output of keto-reacting substances (Table I) amounted to 252 ± 16 mg (S.E.M.) expressed as triketocholanic acid, or 52.6 ± 4.4 mg of carbonyl groups ($252 \times .209 = 52.6$). The daily keto output was subject to considerable individual variation under basal conditions, so that the output of keto-reacting substances ranged from 98 to 507 mg per 24 hours in the different dogs. One of the dogs showed a 50% variation between different control periods; the others varied only from 5 to 15%.

Output of keto-reacting substances when natural ox-bile salts were administered. When natural ox-bile salts were administered, either in 3 or 5 g daily doses, there was an increase in keto groups secreted in the bile, *i. e.*, 72.1 mg and 92.8 mg respectively as compared to 52.6 mg for "control" bile (Table I). The source of the increased keto-reacting substances in bile is problematic. However, as has been shown before and confirmed in this work, when unoxidized cholates are given about 10% are "lost" during one enterohepatic circuit. This loss of 10% is approximately balanced by the increase of 20 mg of keto groups (Table I). This is true only when it is assumed that the natural conjugated bile acids "lost" are changed to oxidized conjugated bile acids which contain 11.2% keto groups. This is reasonable because if the natural conjugated bile acids in cattle bile are oxidized they contain 11.2% keto groups.

TABLE I.
Effect of Unoxidized and Oxidized Bile Acids on Content of Keto-reacting Substances in Dog's Bile.

Procedure	No. of dogs	No. of tests	Bile, cc/24 hr	Total C = O groups, mg/24 hr	Increase C = O groups over control	Recov. of keto acid fed	% recov.
Control: Diet without bile salts	15	55	126	52.6			
1.5 g cholic acid as cattle bile*	9	34	190	72.1	19.5		
3.0 g oxidized conjugated bile salts as they occur in cattle bile. 11.2% C = O	11	14	175	107.2	54.6†	488‡	16§
3.0 g ketocholanic acid from cattle bile. 18.3% C = O	7	10	251	186.2	133.6	730	24
3.0 g dehydrocholic acid. 20.9% C = O	6	10	264	227.3	174.7	836	28

*Contains no keto-reacting substances.
†107.2 — 52.6 = 54.6.
‡ 54.6 ÷ 11.2 × 100 = 488.
133.6 ÷ 18.3 × 100 = 730.
§488 ÷ 3000 = 16%. Note that the per cent recovered is about proportional to the keto acid content of the oxidized bile acids fed.

Output of keto-reacting substances when oxidized or ketocholanic acids were administered. We used a product containing oxidized conjugated bile acids made from cattle bile (Dechacid No. 14, Wilson Laboratories, Chicago). That is, cattle bile was oxidized without splitting-off the taurine and glycine. This product contained by analysis 11.2% keto or carbonyl groups. We used another product containing oxidized unconjugated bile acids made from cattle bile (Ketochol, Searle, Chicago). This product contained by analysis 18.3% keto or carbonyl groups. We also used pure dehydrocholic acid (Decholin, Riedel-de Haen, New York), which contained by analysis 20.9% carbonyl groups.

When the oxidized bile acids were fed a marked increase in the keto-reacting substances in the bile of the dogs resulted (Table I). By subtracting the control output of keto-reacting substances from the total output, one may obtain the increase due to the keto-acids fed. This increase may be assumed to be the amount of keto-acid fed that is recovered in the bile. In this way the recovery of the keto acid fed may be estimated. Though two other assumptions are possible, we believe the one mentioned is the most logical, since the increase in keto-reacting substances in bile after unoxidized bile acids are fed appears to be due to oxidation of the bile acid lost during an enterohepatic circuit rather than to choleresis *per se.*

Conclusions. (1) Keto-reacting substances are present in dogs' bile and can be quantitatively determined. (2) The output of keto-reacting substances is increased slightly when unoxidized bile acids are fed. (3) When oxidized bile acids or ketocholanic acids are administered orally, the output of keto-reacting substances in the bile is markedly increased. By assuming that the increase over the control output is due to the ketocholanic acid fed, one may calculate the recovery in the bile of the ketocholanic acid administered.